

*Annual Review of Analytical Chemistry*  
Electrochemical Affinity  
Assays/Sensors: Brief  
History and Current Status

Kenneth R. Wehmeyer,<sup>1</sup> Ryan J. White,<sup>1,2</sup>  
Peter T. Kissinger,<sup>3</sup> and William R. Heineman<sup>1</sup>

<sup>1</sup>Department of Chemistry, University of Cincinnati, Cincinnati, Ohio 45221-0172, USA;  
email: kenwehmeyer@yahoo.com, white2r2@ucmail.uc.edu, heinemwr@ucmail.uc.edu

<sup>2</sup>Department of Electrical Engineering and Computer Science, University of Cincinnati,  
Cincinnati, Ohio 45221-0030, USA

<sup>3</sup>Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, USA;  
email: petekissinger@gmail.com

Annu. Rev. Anal. Chem. 2021. 14:109–31

The *Annual Review of Analytical Chemistry* is online at  
anchem.annualreviews.org

<https://doi.org/10.1146/annurev-anchem-061417-125655>

Copyright © 2021 by Annual Reviews.  
All rights reserved

### Keywords

electrochemical, affinity assay, immunoassay, aptamer, sensor, biosensor, immunosensor, ligand binding, nanoparticle

### Abstract

The advent of electrochemical affinity assays and sensors evolved from pioneering efforts in the 1970s to broaden the field of analytes accessible to the selective and sensitive performance of electrochemical detection. The foundation of electrochemical affinity assays/sensors is the specific capture of an analyte by an affinity element and the subsequent transduction of this event into a measurable signal. This review briefly covers the early development of affinity assays and then focuses on advances in the past decade. During this time, progress on electroactive labels, including the use of nanoparticles, quantum dots, organic and organometallic redox compounds, and enzymes with amplification schemes, has led to significant improvements in sensitivity. The emergence of nanomaterials along with microfabrication and microfluidics technology enabled research pathways that couple the ease of use of electrochemical detection for the development of devices that are more user friendly, disposable, and employable, such as lab-on-a-chip, paper, and wearable sensors.

ANNUAL  
REVIEWS **CONNECT**

[www.annualreviews.org](http://www.annualreviews.org)

- Download figures
- Navigate cited references
- Keyword search
- Explore related articles
- Share via email or social media

## 1. INTRODUCTION AND BRIEF HISTORY

Immunoassays, and other ligand-binding techniques, are a mainstay for the trace analysis of a wide range of analytes, including drugs, toxins, endogenous biomarkers, proteins, DNA/RNA, pesticides, pathogenic bacteria, viruses, and specific cell types across a diverse array of interests (1–3). The importance of immunoassays becomes apparent given that the global market in 2018 was US\$18 billion and is projected to reach ~\$27 billion by 2024 (4, 5). Ligand-binding assays are based on a highly selective interaction between an affinity element (an antibody for immunoassays) and the target analyte. Typically, a labeling scheme is required to enable the detection of the target analyte.

In the late 1950s, Berson & Yalow (6) ignited the field with the development of radioimmunoassay using antibodies as the affinity element and radioisotopes as the signaling label for the determination of insulin. Radioactive labels provided the inherent sensitivity of isotopic counting techniques and low background interference, but concerns arose from safety, disposal, and training issues.

The success of radioimmunoassay led to an explosion of research into alternate labeling schemes, including optical (7), electronic (8), piezoelectric (9), gravimetric (10), and electrochemical (11–13). The first breakthrough came in 1971 with Engvall & Perlmann's (14) use of enzymatic conversion of a nondetectable substrate into an optically detectable product. When combined with a rinsing step after antibody capture, this provided sensitivity and selectivity rivaling radioimmunoassay. Their enzyme-linked immunosorbent assay (ELISA) is currently the most successful assay format, with over 500,000 publications and widely available commercial kits for hundreds of analytes from many commercial vendors. Yet, even given its great success, ELISA has limitations, including the achievable detection limits with a single enzyme, multiple processing steps, and the frequent requirement of a relatively sophisticated laboratory to implement the assays (15).

Electrochemical detection techniques offered an attractive alternative due to inherent sensitivity, small sample size requirements, relatively low cost of instrumentation, and ease of use, allowing widespread application outside of central laboratories, miniaturization for development of portable sensors, and multianalyte detection (16, 17). Historically, Breyer & Radcliff's (18) use of polarography in 1951 to qualitatively investigate antibody-analyte interactions predated the development of radioimmunoassay. Quantitative application of electrochemical immunoassays was achieved in the 1970s by several research groups. Heineman, Anderson, and Halsall (19) used a mercury acetate redox label together with differential pulse polarography at a dropping mercury electrode to minimize difficulties with adsorption of protein on the electrode for the determination of estriol. Weber & Purdy (20) employed voltammetry for the determination of electrochemically active morphine and also used a ferrocenyl redox label. The Heineman/Halsall group followed quickly with enzyme labels for phenytoin, digoxin, and orosomucoid protein, as well as a metal label for human serum albumin that improved the detection limit by using stripping voltammetry, the most sensitive electrochemical technique at the time (21–24). Building off of Clark & Lyons's 1962 groundbreaking electrochemical biosensor for glucose (25), Aizawa and colleagues (26) developed an electrochemical enzyme immunosensor using membrane-bound antibody and a catalase-labeled analyte for the detection of immunoglobulin G (IgG). Mattiasson & Nilsson (27) reported an enzyme immunoelectrode using catalase and glucose oxidase labels for sensing human serum albumin and insulin. Concurrently, Janata (28), Yamamoto et al. (29), and Alexander & Rechnitz (30) evaluated potentiometric approaches for immunoassay and ligand-binding sensors. These efforts expanded the capabilities of electroanalytical chemistry, allowing the determination of nonelectroactive analytes. Electrochemistry's amenability to measurements of very small volumes, enzyme amplification, novel interdigitated electrodes, and reduction of

Downloaded from [www.annualreviews.org](http://www.annualreviews.org).

Guest (guest)

IP: 3.147.8.145

On: Sun, 30 Jun 2024 15:26:52

nonspecific adsorption were exploited to steadily push down the limits of detection into the attomole levels by the early 2000s (31, 32). Although both potentiometric and voltammetric modes have been pursued, assays based on current measurements have been more widely employed.

The early work of these pioneers catalyzed an explosion of research applying electrochemical detection approaches for immunoassay, generating over 11,000 publications, with half of those occurring between 2010 and 2020. This encompassed multiple research groups and a wide variety of analyte classes (33–35). Additional affinity elements such as aptamers (36), molecularly imprinted polymers (MIPs) (37), DNA/RNA (38), and lectins (39) provided new vectors for specificity. A variety of labeling/signal amplification approaches, including noble metals (40), bimetallic and metal inorganics (41), polymeric enzymes (42), nanozymes (41), DNA-active enzymes (DNAzymes) (43), DNA hybridization amplification approaches (44), and new redox labels (45), were investigated. Additional electrochemical detection schemes have been applied to increase assay format flexibility and sensitivity (46). Along with these new approaches, there have also been developments in micromachined and micrototal analysis systems ( $\mu$ TAS) (31) and new electrode formats such as screen-printed electrodes (47) and interdigitated array electrodes (48), and the introduction of nanomaterials to increase assay flexibility, selectivity, and sensitivity (49, 50).

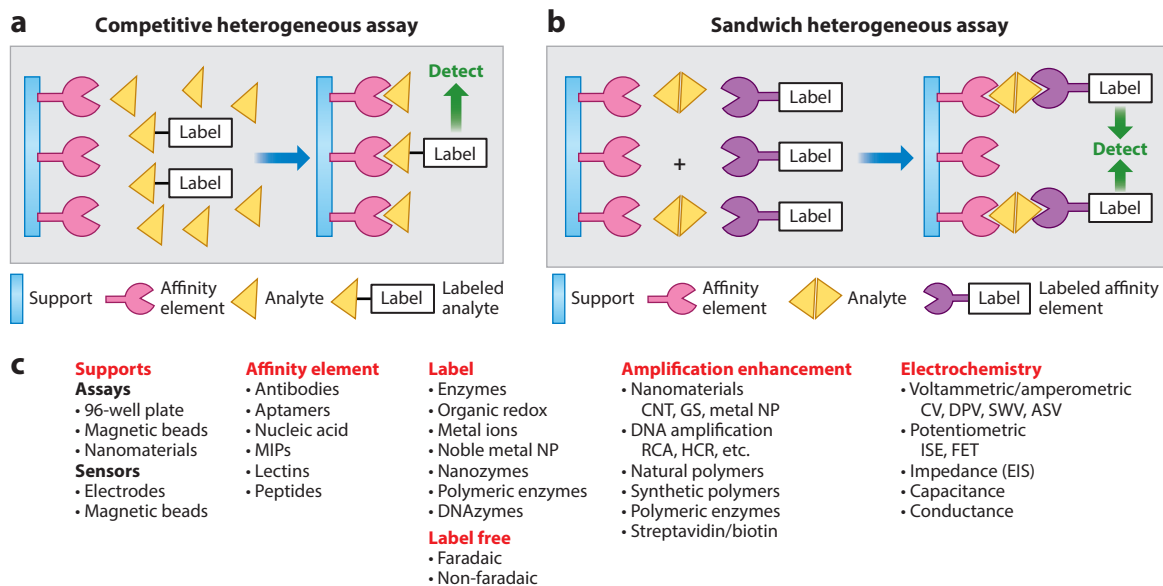
Only representative examples of key developments are given in this short review. We briefly present ligand-binding assay formats, affinity elements, and electrochemical detection methods. Applications of the most widely employed labeling/signal amplification schemes are reviewed for both assay and sensor formats. The application of nanomaterials, multianalyte detection, and micromachined devices is highlighted. Commercial applications and future areas of focus are assessed.

## 2. ELECTROCHEMICAL AFFINITY ASSAYS/SENSORS, AFFINITY ELEMENTS, AND IMMOBILIZATION APPROACHES

A brief discussion of electrochemical affinity assays/sensors, affinity elements, and immobilization approaches for affinity elements is given below. The interested reader is referred to more in-depth descriptions of these areas (51, 52).

### 2.1. Electrochemical Affinity Assays/Sensors

The heart of an affinity assay/sensor is the specific and strong interaction of the affinity element with the target analyte (**Figure 1**). The lack of a strong measurable signal from the binding event itself often necessitates the use of a labeling scheme to facilitate the detection. Electrochemical affinity assays can be done in a homogeneous format requiring no separation step prior to detection or in a heterogeneous format where a solid support is used for attaching the affinity element or analyte to enable isolation from the sample matrix. The heterogeneous formats are the most popular since they are typically simpler and often enable lower limits of detection. Additionally, selectivity and sensitivity are improved because potential interfering sample components are rinsed away. Heterogeneous assays are done in two major formats, the sandwich format involving the use of two affinity elements and the competitive format using one (**Figure 1**). In the sandwich format, an affinity element is attached to solid support (e.g., a plastic tube, magnetic beads, nanomaterials, or an electrode) and incubated with the analyte to isolate it from the sample matrix. A second affinity element containing the detection label (e.g., enzyme, metal, redox couple, or DNA amplification probe) is incubated with the isolated analyte-bound affinity element to form the sandwich complex. The sandwich format is restricted to large analytes such as proteins and cells, as two unique binding sites must exist on the analyte. In one popular competitive assay format, a solid-phase analyte and analyte in the sample solution compete for a limited amount of



**Figure 1**

Scheme for heterogeneous affinity assays/sensors. (a) Competitive format and (b) sandwich format. (c) Summary of supports, affinity elements, labels, amplification approaches, and electrochemical detection techniques. Abbreviations: ASV, anodic stripping voltammetry; CNT, carbon nanotube; CV, cyclic voltammetry; DPV, differential pulse voltammetry; EIS, electrochemical impedance spectroscopy; FET, field-effect transistor; GS, graphene sheet; HCR, hybridization chain reaction; ISE, ion-selective electrode; MIP, molecularly imprinted polymer; NP, nanoparticle; RCA, rolling circle amplification; SWV, square wave voltammetry.

labeled affinity element. In another format an analyte and a labeled analyte in solution compete for a limited amount of solid-phase affinity element (**Figure 1**). The competitive heterogeneous assay format is applicable to both small and large molecular weight analytes because only a single binding interaction site is required.

The key distinguishing feature for a sensor versus an assay is that the sensor should be a completely self-contained device where the affinity element or analyte is attached directly to the electrode surface and the electrode serves as the transducer for providing a signal proportional to the analyte concentration as a result of the interaction (**Figure 1**). The need for measuring analytes in point-of-care or point-of-use applications in medical, environmental, food, and public safety has been the main driving force behind the development of reliable, easy-to-use, and relatively inexpensive electrochemical affinity sensors (45).

## 2.2. Affinity Elements

A variety of affinity elements have been employed in electrochemical affinity assay/sensor applications, including antibodies, aptamers, nucleic acid, lectins, and MIPs (36, 53–56). The first and most widely employed affinity element is antibodies. Antibodies, most typically IgG, are produced as part of the immune response in mammals and provide selectivity and strong binding ( $K_d = 10^{-8} - 10^{-10}$  M) to the target analytes. Aptamers, synthetic single-stranded DNA or RNA sequences produced by a combinatorial chemistry process, are more recently used affinity elements. Aptamers can assume three-dimensional (3D) structures that allow them to bind to target analytes with similar binding properties as antibodies but offering greater stability, lower cost, ease of production, and less lot-to-lot variability compared to antibodies (53). Moreover, aptamers

can be developed or designed to undergo a conformation change upon analyte binding that enables detection of small-molecule targets (<1,000 mol wt) that are often difficult to quantify via antibody-based sensors. MIP films are prepared by copolymerizing functional monomers and a crosslinker in the presence of the analyte of interest as the template for selective binding. Finally, other biological materials, such as lectins, carbohydrates, and certain peptides, have been used in more limited applications to specific analyte classes.

### 2.3. Affinity Element Immobilization and Nonspecific Binding

The attachment of affinity elements to the solid surface in a reproducible manner with the correct binding orientation plays a critical role in determining the selectivity and sensitivity of affinity assays/sensors, and a number of immobilization approaches have been used (57). Physical adsorption of the affinity element has been a popular approach owing to its simplicity, but it provides little to no control over orientation and is not irreversible. Chemical attachment through functional groups on the affinity element and the solid surfaces offers a permanent attachment and provides some control over orientation. The use of protein A for affinity attachment of IgG to a surface offers a high degree of control over orientation. A more general approach for controlling orientation, applicable to any affinity element, is the biotin-streptavidin coupling ( $K_d = 10^{-15}$  M). Streptavidin is typically attached to the solid surface, and biotin is attached to the affinity element and/or the label, providing a generic approach. Nucleic acid recognition elements lend themselves to surface attachment through chemical modification of the phosphate backbone via phosphoramidite chemistry. Addition of thiol groups to either the 3' or 5' terminus allows self-assembled monolayer formation on gold surfaces (58). Other chemical modifications to the phosphate backbone, including azides and alkynes, enable click chemistry functionality to carbon electrode surfaces. Finally, entrapment of the affinity element within a polymer matrix by copolymerization can be used with a mixture of monomers or through electrostatic binding with a given polymer substrate. Nonspecific binding of endogenous sample matrix components and the labeling constructs can lead to high background signals in an assay/sensor and thereby a deleterious effect to the limit of detection. To lessen the effect of nonspecific binding on assay performance, a variety of agents are used to passivate the solid phase and electrode surface, including proteins (albumin and casein), polymers, nonionic surfactants, and various surface-assembled monolayer chemistries (59, 60).

## 3. ELECTROCHEMICAL DETECTION TECHNIQUES

Beginning with pioneering work in the 1970s, a wide variety of electrochemical detection techniques have been implemented in affinity assays/sensors. A brief description of these approaches is presented below, but detailed references are available for more in-depth descriptions (61–63). Electrochemical approaches can be broadly classed into four transduction categories: current (amperometry/voltammetry), potential (potentiometric), impedance, and electrochemiluminescence-based technologies. Voltammetry approaches, including cyclic voltammetry, differential pulse voltammetry, square wave voltammetry, and anodic stripping voltammetry, use potential waveforms at a working electrode and measure the responding current. Amperometry is also a current measurement approach that applies a constant potential or potential pulses (intermittent pulse amperometry) for recycling redox-labeled recognition elements (64) and measuring the resulting current over time. Potentiometric techniques are based on monitoring the potential of the electrochemical cell under no or negligible current conditions, as in the measurement of pH with a glass electrode. Potentiometric approaches commonly involve the use of an indicator electrode modified with an affinity element to interact with the analyte or modified to respond to a change in a solution species activity driven by the binding event. Electrochemical impedance spectroscopy

(EIS) is typically done in the faradaic mode using a redox probe such as ferrocyanide/ferricyanide by polarizing the electrode at its formal potential, applying a small-amplitude sinusoidal voltage across a range of frequencies, and measuring the resulting current response. Finally, electrochemiluminescence, pioneered by Bard & Whitesides (65), typically uses a tris-ruthenium(bipyridine) complex  $[\text{Ru}(\text{byp})_3^{2+}]$  oxidized at the electrode. This generates a product that undergoes a high-energy electron transfer reaction with an amine compound, resulting in the emission of a luminescent photon from the excited state of the ruthenium complex. Various working electrode materials have been used, including glassy carbon, carbon paste, noble metals (gold, platinum, and silver), mercury film-modified versions of the preceding electrodes, and indium tin oxide films. Building from early work by Kuwana & Strojek (66) with optically transparent electrodes for spectroelectrochemistry, thick and thin metal films such as screen-printed electrodes, most typically of carbon, gold, or platinum, allow the creation of cost-effective and disposable electrodes versus the traditional electrochemical electrodes.

#### 4. NANOMATERIALS

The significant advances in nanomaterials have had a revolutionary impact on electrochemical affinity assays, especially in the development of sensors (16, 67). It is not possible to comprehensively review the literature on nanomaterials here, so only a few widely applied examples are presented; in-depth treatments are available elsewhere (67–69). Nanomaterials have been used as carriers to increase the loading of affinity elements, as carriers to increase the loading of labels, as amplification platforms, and as direct and indirect electroactive labels (2). Nanomaterials provide a large surface area for these applications and thus contribute to improved detection limits while also improving the accessibility of the recognition elements to target analytes (70–72). Materials used as nanomaterials include carbon nanotubes, both the single-wall and multiwall graphene sheets, reduced graphene, and graphene oxide/reduced graphene oxide (2, 69, 73). Other carrier materials include nanoparticles (NPs) of noble metals (3, 40), which are mesoporous core shell NPs where detection labels can be incorporated into the pores and capped for later release (74). Natural/synthetic polymer nanomaterials, including conducting polymer (75), hydrophobic gels (76), branched synthetic polymeric dendrimers, and biopolymers such as DNA, have been used as carriers (4). The use of DNA amplification schemes, for example, is based on the concept of creating long stretches of either single- or double-stranded DNA to serve as a scaffold for attaching large quantities of electroactive labels such as organic redox labels (77), metal labels (78), and DNAzymes (79). Nanomaterial labels include noble metal NPs, colloidal Au, bimetallic NPs, and metal inorganic quantum dots (QDs) that can serve as redox labels directly or in some cases after dissolution with acid (3, 15, 40, 41). Certain nanomaterials possess peroxidase-like activity (e.g., noble metal NPs, bimetallic NPs, Pd NPs, QDs,  $\text{Fe}_3\text{O}_4$  NPs) and can serve as alternatives to natural enzymes (3, 40, 41). Additionally, polymeric enzyme particles have been employed as a labeling strategy (42).

#### 5. ELECTROCHEMICAL AFFINITY ASSAYS/SENSORS: LABEL FOCUS

Research interest in electrochemical affinity assays/sensors has grown to include multiple analyte classes and approaches too numerous to cover in this short review. There are reviews of environmental detection (80, 81), clinical diagnostics (82), toxin detection (83), clinical biomarker screening (84), pathogen/virus detection (85, 86), food safety (87, 88), and cancer diagnosis (16, 89–91). Common themes in the past decade have been to quantitate lower analyte levels, as well as to develop rugged, reliable, and cost-effective devices for point-of-care and point-of-use applications in

medical, food, and homeland security areas. In response, various labeling and signal amplification schemes have been developed, including the use of nanomaterials.

### 5.1. Metal Label Electrochemical Affinity Assays/Sensors

Since their inception (21), metal ion labels have become an increasingly popular approach for multianalyte electrochemical affinity assays/sensors (92–95). Additional new metal-based detection schemes include the use of noble metal NPs and QDs (95–100). Compared to the first metal labels (21) that released one metal ion per captured molecule, these metal labels release hundreds of thousands to millions of ions, providing a large amplification factor leading to improved sensitivity. Very importantly for some applications, metals are more stable than enzymes, which also provide amplification.

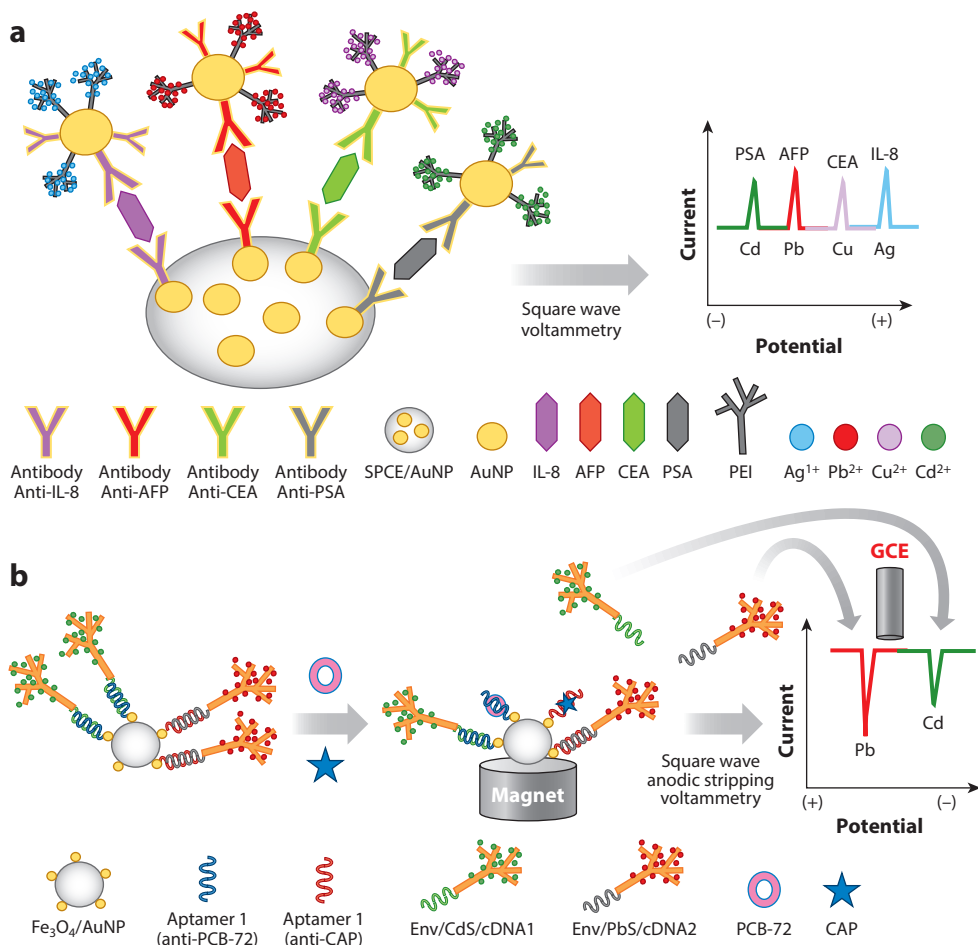
A sandwich-based immunosensor for the simultaneous measurement of four cancer biomarkers [alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), prostate-specific antigen (PSA), and interleukin 8 (IL-8)] in human serum using multiple metal labels is shown in **Figure 2a** (93). The immunosensor used a single screen-printed carbon electrode and modified gold nanoparticles (AuNPs) containing the capture antibody for each of the markers. Four separate polyethyleneimine-coated AuNPs (PEI/AuNPs), each containing a separate covalently attached secondary antibody and carrying a unique metal ion label ( $\text{Cd}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Cu}^{2+}$ , or  $\text{Ag}^{1+}$ ) chelated to the PEI/AuNPs, were used as the labeling scaffold. Incubation of the immunosensor with the analytes or with spiked human serum followed by incubation with the labeling scaffold resulted in the final analysis complex (**Figure 2a**). The immunosensor was transferred to a buffer solution and measured with square wave voltammetry for the simultaneous detection of the four analytes.

Special mention is owed to the use of metal label complexes, especially  $\text{Ru}(\text{byp})_3^{2+}$ , in electrochemiluminescence immunoassays. Roche Diagnostics Elecsys and Meso Scale Discovery have both successfully commercialized the electrochemiluminescence immunoassay for single- and multianalyte analysis for a wide variety of biomolecules (101).

QDs have been coupled with other nanomaterials for sensitive and multianalyte assays (96–98, 100, 102, 103), which has been recently reviewed (99). An aptamer-based electrochemical affinity assay (**Figure 2b**) developed for the simultaneous detection of an environmental pollutant [polychlorinated biphenyl-72 (PCB72)] and an antibiotic drug (chloroamphenicol) in fish tissue is a representative example (96). The two ligand-specific aptamers were attached to the same  $\text{Fe}_3\text{O}_4$  magnetic beads coated with AuNP to give the binding scaffold. The labeling scaffold was composed of QD labels, CdS for PCB72, and PbS for chloroamphenicol, attached to separate dendritic commercial EnVision polymer NPs. The EnVision particles also contained a single-stranded complementary DNA (cDNA) sequence that specifically binds to its corresponding aptamer. The incubation of the binding scaffold with the labeling scaffold yielded the completed analysis complex formed by the cDNA pairing with their respective aptamers. Incubation of the detection complexes with the analyte standards or extracts of spiked fish samples displaced the labeling scaffolds from the beads. After removal of the magnetic beads with a magnet, the displaced labeling scaffolds in solution were treated with acid, and the metals were measured by square wave stripping voltammetry at a glassy carbon electrode.

### 5.2. Organic and Organometallic Redox Labels

Organic and organometallic redox labels for affinity assays/sensors continue to be widely used for sensitive and multianalyte analysis (104–109). A novel homogeneous electrochemical affinity assay was developed for the measurement of an algal biotoxin, brevetoxin B ( $\text{PbTx-2}$ ), using methylene



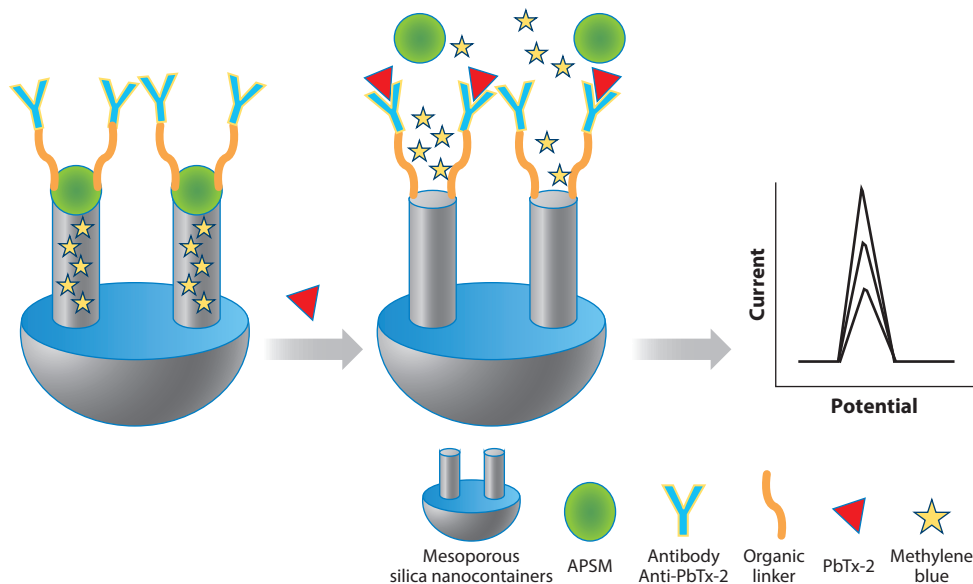
**Figure 2**

(a) Electrochemical sandwich immunosensor for the simultaneous measurement of four cancer biomarkers (93). (b) Electrochemical aptamer-based assay for the detection of an environmental pollutant (PCB-72) and an antibiotic (CAP) in fish tissue (95). Abbreviations: AFP, alpha-fetoprotein; AuNP, gold nanoparticle; CAP, chloroamphenicol; cDNA complementary DNA; CEA, carcinoembryonic antigen; Env, EnVision polymer particle; GCE, glassy carbon electrode; IL-8, interleukin 8; PEI, polyethyleneimine; PSA, prostate-specific antigen; SPCE, screen-printed carbon electrode.

blue (MB) redox labels loaded into mesoporous silica nanocontainers (MSNs) (108). The detection probe consisted of a monoclonal antibody attached to the MB-loaded MSNs that were sealed with animated polystyrene microspheres (APSMs) to give the completed complex. Binding of PbTx-2 with the antibody resulted in displacement of the APSM from the pores and the release of MB from the nanocontainers into the solution (Figure 3). The MB was detected at an indium tin oxide electrode using square wave voltammetry without a separation step.

Structure-switching, or folding-based sensors, employing nucleic acids were introduced by Plaxco and coworkers in 2003 (110) using stem-loop DNA and later using aptamers in 2005 (111). These utilize organic or organometallic redox-labeled recognition elements such as MB, ferrocene, or anthraquinone labels (although predominately MB). Faradaic current from the





**Figure 3**

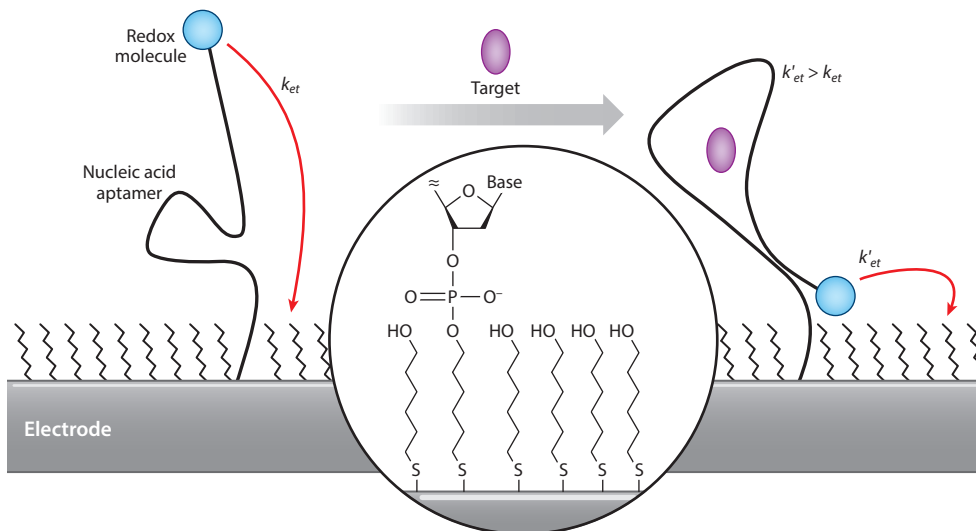
An electrochemical homogeneous immunoassay for brevetoxin B (PbTx-2) using mesoporous silica nanocontainers loaded with methylene blue held in pores by animated polystyrene microspheres (APSMs) (108).

reversible reduction of the redox label provides a signal that changes with a target-induced conformation change of the nucleic acid or peptide (64) (**Figure 4**). The conformation change affects the charge transfer rate between the appended redox label and the electrode surface (110, 112), typically monitored using voltammetric methods such as square wave voltammetry (113). More recently, studies using amperometry (114) and intermittent pulse amperometry (64) demonstrate the improved temporal resolution of this class of affinity-based sensor.

### 5.3. Enzyme Labels

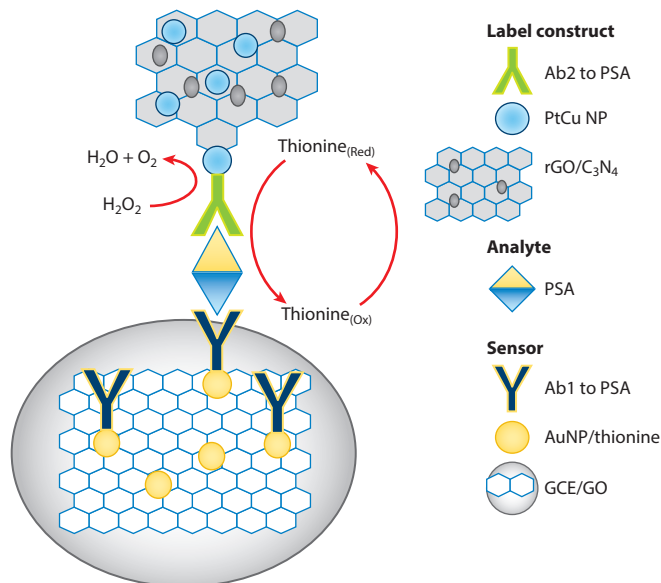
Enzyme labels remain one of the most popular approaches for affinity assays/sensors. A recent trend improves the signal amplification capabilities by using polymeric enzymes and NPs to increase enzyme loading. Additionally, artificial enzymes, so-called nanozymes, have been used as a replacement for natural enzymes to improve assay cost and reliability (115–123).

Nanozymes are NPs of noble metals (Au, Pt, Ag), bimetallic particles (e.g., PtPd, PtCu), and iron-based ( $\text{Fe}_3\text{O}_4$ ) particles possessing peroxidase-like activity. They offer advantages versus biological enzymes in terms of cost, stability, and batch-to-batch consistency but typically do not possess as high a substrate turnover capability (3). A sandwich-based affinity sensor for the cancer biomarker PSA was designed using a glassy carbon electrode–modified AuNP, thionine, graphene oxide, and a capture antibody (124) (**Figure 5**). The labeling scaffold was a reduced graphene oxide/graphitic carbon nitride (rGO/ $\text{C}_3\text{N}_4$ ) construct with the secondary antibody and loaded with a PtCu NP. Incubation of the sensor with PSA followed by a labeling scaffold resulted in the detection complex. Upon addition of  $\text{H}_2\text{O}_2$  the PtCu NP acted as a peroxidase reducing the  $\text{H}_2\text{O}_2$  and oxidizing the thionine attached to the reduced graphene oxide. The oxidized thionine was then reduced at the glassy carbon electrode using differential pulse voltammetry.



**Figure 4**

Electrochemical aptamer-based sensors employ redox-labeled nucleic acid aptamers to provide specific recognition capabilities. The sensing mechanism is based on a reversible target-induced conformation change of the electrode bound aptamer that alters the charge transfer rate between the redox marker and the electrode. This change in charge transfer rate results in a change in faradaic current that serves as the basis for sensor signaling. Abbreviation:  $k_{et}$ , charge transfer rate. Figure adapted with permission from Reference 64; copyright 2018 American Chemical Society.



**Figure 5**

A sandwich electrochemical immunosensor for the cancer biomarker PSA. Abbreviations: Ab, antibody; GCE/GO, glassy carbon electrode/graphene oxide; NP, nanoparticle; PSA, prostate-specific antigen; rGO/C<sub>3</sub>N<sub>4</sub>, reduced graphene oxide/graphitic carbon nitride (124).

Downloaded from www.annualreviews.org.

Guest (guest)

IP: 3.147.8.145

On: Sun, 30 Jun 2024 15:26:52

## 6. ELECTROCHEMICAL LIGAND-BINDING ASSAYS AND SENSORS: LABEL-FREE

Label-free approaches offer the advantage of often being less complex than label-based approaches, but often they are not as sensitive. The major electrochemical-based approaches are based on potentiometric, impedance (e.g., EIS), conductimetric, and capacitance detection modes (125). The EIS approach has been investigated most extensively in the faradaic mode with a redox probe, typically potassium ferrocyanide/ferricyanide (85, 86, 126–129). An example is a highly sensitive electrochemical immunosensor for glial fibrillary acidic protein, a biomarker of central nervous system injury, using faradaic EIS detection with a potassium ferrocyanide/ferricyanide couple as the redox probe. Faradaic EIS approaches have been used for the detection of viruses and antibodies to viruses (85, 86, 126).

## 7. DEVICES: MICROCHIP, LAB-ON-A-CHIP, PAPER, AND WEARABLE SENSORS

In addition to progress in the chemistry side of electrochemical affinity assays/sensors, significant advances have also been made in the device side. The emergence of new technologies such as microfabrication and microfluidics opened new research pathways that enabled the ease-of-use benefits of electrochemical affinity assays/sensors to be exploited for the development of more user-friendly, disposable, microfabricated, lab-on-a-chip, paper, and now, even wearable electrochemical affinity assays/sensors.

Aside from the pioneering immunosensor from the Aizawa group in 1976 (26), early research focused primarily on immunoassays with electrochemical detection (23). The emergence of sensors began by attaching the capture Ab directly to the detector electrode. A number of strategies for implementing this approach were explored (130), most of which involved removing the electrode after the capture step for rinsing or adding reagents for the detection step. Although these were not completely independent sensors such as the pH electrode, they constituted a major step in immunosensor development. Also, because Ab–Ag dissociation reactions are slow and dissociation is required to reset the sensor/electrode for reuse, sensors were generally single-use, disposable devices. However, electrodes at the time were rather expensive to dispose of after only a single use. Technologies such as screen printing and microfabrication changed this by providing cheaper, disposable electrodes. Also, increasingly complex electrodes could be fabricated, such as interdigitated array electrodes that improved sensitivity by redox cycling (48). Their potential impact is clearly illustrated by analogy to the evolution of the electrochemical biosensor for glucose measurement using microfabricated electrodes, where commercially viable sensors are now mass produced for single-use applications at sufficiently low cost and with adequate reproducibility for personal use without calibration for the huge diabetes market.

The emergence of other microtechnology—microfluidics, microelectromechanical systems (MEMS), micrototal analysis systems (TAS), lab-on-a-chip (131)—stimulated development of miniaturized sensor systems that would carry out all of the steps of an immunoassay that enable it to be so selective and sensitive (132–135). Magnetic bead-based electrochemical immunoassays based on the detection of enzyme-generated *p*-aminophenol were developed and evaluated for monitoring the US water supply by using representative toxin, virus, spore, and bacterium harmless simulants. The assays were demonstrated to work on 20 samples of drinking water collected from around the United States and spiked with the simulant agents (136). Since that time, a number of formats combined a variety of labeling schemes and the use of nanomaterials and magnetic beads (98, 115, 122, 137–142).

A recent example is a lab-on-a-membrane competitive electrochemical immunosensor for the simultaneous determination of bovine casein (bCN) and bovine IgG (bIgG) using a nylon

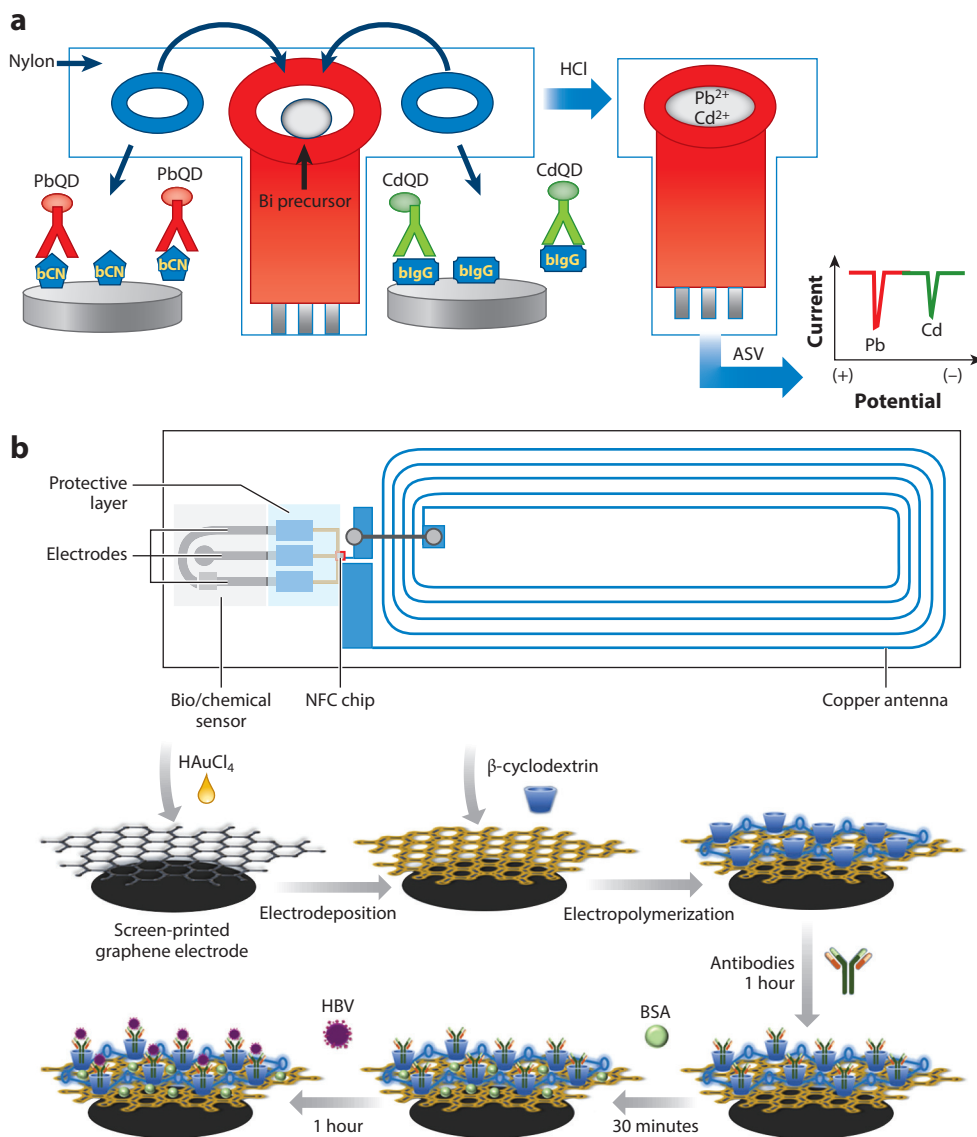
membrane with two symmetrical screen-printed assays zones and a center zone containing a bismuth citrate-loaded graphite electrode (98) (**Figure 6a**). One assay zone has bCN analyte attached to the membrane, and the other assay zone has bIgG attached to the membrane. A drop of sample is added to each assay zone and then incubated with their respective biotinylated antibodies. Following washing, streptavidin-conjugated PbS-QDs are added to the bCN, and streptavidin CdS-QDs are added to the bIgG zone. Following incubation and washing, acid is added to both zones to release the metal ions,  $\text{Pb}^{2+}$  and  $\text{Cd}^{2+}$ , for measurement by anodic stripping voltammetry after folding the assay zones onto the electrode zone in the center. Another membrane-based microfluidic approach was applied for the determination of the cancer biomarker VEGF165 (vascular endothelial growth factor 165) using a porous polycarbonate membrane with covalently attached capture antibodies placed over the top of micro gold electrodes as part of a microfluidic cell (129). The analyte in undiluted serum was captured in the microfluidic chip and then detected using a secondary antibody labeled with alkaline phosphatase enzyme, where the biotin-streptavidin approach was used to couple the enzyme to the secondary antibody and the enzyme product, *p*-aminophenol, was detected by differential pulse voltammetry.

Microfluidic paper-based analytical devices ( $\mu$ PADs) were pioneered by the Whitesides (143) and Henry groups (145, 146) to achieve low-cost, high-performance devices without the need for external fluid control. An origami paper-based electrochemical immunosensor for an inflammatory biomarker, C-reactive protein, was developed using a screen-printed carbon electrode modified with graphene, an AuNP containing the antibody, and faradaic EIS detection (139). A paper-based device for use with peptide-selective aptamers and an initiator DNA strand were coupled with the hybridization chain reaction amplification approach to enable the binding of a  $\text{Ru}(\text{phen})_3^{2+}$  label to the double-stranded DNA grooves for electrochemiluminescence detection (142).

A simple, card-sized, label-free electrochemical immunosensor consisting of an antibody-modified electrode with near-field communication controlled by a smartphone (**Figure 6b**) has been used to detect viruses (144). The top drawing of the card shows the electrochemical immunosensor connected to the near-field communication chip potentiostat and the antenna for cell phone communication. The schematic illustrates fabrication of the biosensor by modifying a screen-printed graphene electrode with AuNPs to increase sensitivity, electropolymerize  $\beta$ -cyclodextrin on the surface for binding capture antibody, and add bovine serum albumin (BSA) to minimize nonspecific adsorption from the sample. The last step is to capture the virus from the sample for detection. Hepatitis B surface antigen (HBsAg) was quantified using amperometry to measure the current from the  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  redox couple before and after addition of HBsAg. The immunosensor was then demonstrated with chronic hepatitis B virus-infected serum samples.

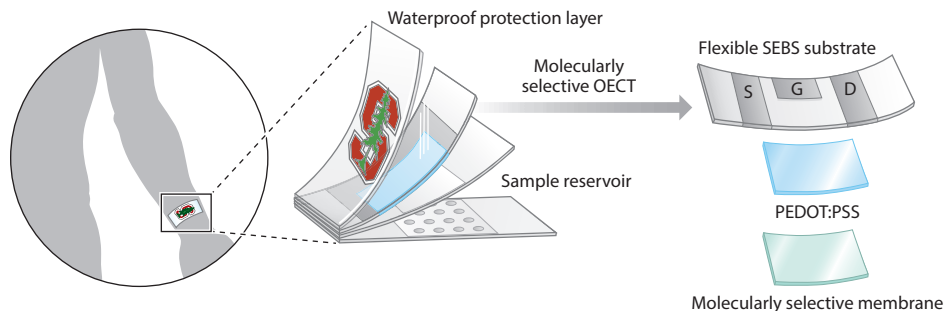
Examples of point-of-use applications that couple electrochemical affinity assays with simple commercial measurement devices include using personal glucose meters for the sensitive detection of a bacterial pathogen (138), a pH meter to detect a cardiac biomarker (141) and food pathogens (147), and a digital multimeter to detect a cancer biomarker (148). These all have coupled enzyme labels and various nanomaterials or magnetic beads to develop sensitive and simple assays. The use of commercial devices has allowed easy application for point-of-use needs with minimal investment.

The age of the wearable or small carried sensors is upon us, and affinity sensors have the specificity and sensitivity to provide the way for measuring biomarkers of the human condition and drugs (149) using noninvasive sample matrices such as sweat, saliva, and urine (150, 151). Sensors have been developed for a range of analytes using antibodies (cytokines and cortisol in sweat and human serum albumin in urine), aptamers (tumor necrosis factor alpha in sweat), MIPs



**Figure 6**

(a) Electrochemical competitive heterogeneous immunosensor for the simultaneous determination of bovine casein (bCN) and bovine IgG (bIgG) on a nylon membrane with quantum dot (QD) labels using a bismuth-modified graphite electrode. Panel adapted with permission from Reference 98; copyright 2016 American Chemical Society. (b) Portable amperometric immunosensor with NFC-Potentiostat chip and schematic illustration of biosensor preparation and capturing virus prior to the detection step. Other abbreviations: ASV, anodic stripping voltammetry; BSA, bovine serum albumin; HBV, hepatitis B virus; NFC, near-field communication. Panel adapted with permission from Reference 144; copyright 2021 Elsevier.



**Figure 7**

Wearable electrochemical immunosensor for cortisol measurement in sweat using a molecularly imprinted polymer-based molecularly selective-organic electrochemical transistor (OECT). Other abbreviations: PEDOT:PSS, polymeric material (poly(ethylenedioxythiophene):poly(styrenesulfonate)); SEBS, styrene-ethylene-butylene-styrene. Figure adapted with permission from Reference 153; copyright 2018 AAAS.

(cortisol), antimicrobial peptides (bacteria on tooth enamel), and lectin (carbohydrate-binding proteins in sweat) (152). A wearable MIP-based sensor scheme for cortisol monitoring in sweat is shown in **Figure 7** (153). The sensor is based on an organic electrochemical transistor (OECT) polymeric material [poly(3,4-ethylenedioxythiophene):poly(styrene sulfonate)] (PEDOT:PSS) with an Ag/AgCl gate transducer covered with a nanoporous cortisol MIP membrane to create a molecularly selective membrane between the OECT and the sweat on the skin to form a molecularly selective OECT (MS-OECT). The sensor construct was produced on an elastomer substrate (styrene-ethylene-butylene-styrene) for flexibility when applied to the skin. The membrane is permeable to ions in the absence of cortisol, resulting in a large change in source-drain current with gating of the OECT channel. But cortisol binding results in blocking ion movement in the channel and reduces the measured current.

## 8. CURRENT STATUS, TECHNICAL CHALLENGES, AND FUTURE DIRECTIONS

The initial pioneering efforts in electrochemical affinity assays/sensors stimulated interest in a new research pathway for electroanalytical chemistry. This significantly expanded the breadth of applications to include nonelectroactive materials with a selectivity enabling the detection of analytes in extraordinarily complex matrices. It was quickly shown that a long-standing nemesis of bioanalytical chemistry, electrode fouling by proteins and other surfactants, could be overcome and that solid electrodes could be used instead of the cumbersome dropping mercury electrode, the dominant electrode for the previous 40 years since the discovery of polarography by Heyrovsky in 1922 (154). The combination of electrochemical detection, new affinity elements, new redox labels and signal amplification schemes and the incorporation of nanomaterials have enabled truly remarkable selectivity and sensitivity while microfabrication and microfluidics have promoted novel devices. Although the electrochemical affinity assay/sensor techniques use relatively simple configurations, the achieved figures of merit can rival the more expensive and complex gold standard trace analysis technique of high-performance liquid chromatography tandem mass spectrometry. Given the multiple affinity-binding modes, the wealth of labeling schemes, and the new capabilities afforded by nanomaterials, it is safe to say that an electrochemical affinity assay/sensor could be developed for essentially any analyte in any matrix.

Clearly, from a research standpoint, electrochemical affinity assays/sensors have been highly successful, covered in well over 15,000 publications in the past decade across a wide variety of analytes. Of the ligand-binding approaches, the electrochemiluminescence-based assays have generated a sustained and significant impact in the market with the introduction of instrumentation by the Meso Scale Discovery and Roche Diagnostics Elecsys systems. Several companies, including GenMark Diagnostics, Genefluidics, Inc., Binx Health Limited, and Abbott Laboratories, offer electrochemical sensor approaches for point-of-care applications using DNA-based affinity approaches for bacterial identification and disease conditions. Abbott offers the iSTAT handheld electrochemical instrument employing ligand-binding approaches. However, the adoption of electrochemical affinity assays/sensors into the marketplace is still limited when compared to the commercial success of ELISA.

Although electrochemical affinity assay/sensors are often less complicated, less expensive, and somewhat faster than traditional analysis methods, there are areas that require improvement before extensive commercial adoption is likely. Proof-of-concept electrochemical affinity assays/sensors often have relied on manual steps for incubation, washing, and final assay development. Although this approach is fine for establishing proof-of-concept, manual steps are limiting in real-life settings. The use of magnetic beads and the introduction of micromachined/ $\mu$ TAS devices have the potential for simplifying and automating the analysis. Another limiting issue is the lack of long-term sensor stability, often owing to the nature of some of the components, such as antibodies and enzymes. Investigators typically show that sensors maintain stability over the short term, for example, weeks to a month, but they lack longer-term stability information needed for a commercial device. The alternate affinity elements, such as aptamers, carbohydrates, and MIPs, as well as the use of nanozymes, hold the potential for addressing stability issues. Standard 2- to 4-mm electrodes are useful for proof-of-concept studies, but their cost often makes them impractical for point-of-use instrumentation. Screen-printed electrodes offer an economical alternative that opens the potential for truly disposable and cost-effective sensors. Real samples often pose special problems, such as nonspecific adsorption of proteins from biological samples, that can potentially interfere with sensor performance. Long-term deployment of sensors in natural water such as lakes and streams is susceptible to biofilm formation, which can interfere with sensor response. Mechanisms for fouling electrode surfaces and antifouling strategies were recently reviewed (156, 157). Adding a protein such as BSA to block nonspecific adsorption sites, as shown in **Figure 6b** (144), or a functionalized monolayer, as shown in **Figure 4** (64), during biosensor preparation are examples of strategies for minimizing fouling. Many reports have lacked a detailed evaluation of the sensor performance with actual samples. They are often characterized by a brief section at the end of the article, demonstrating the potential of the assay using only a limited number of actual or spiked samples for accuracy and precision. By comparison, in a recent publication (155) on the validation of an electrochemical immunosensor, the authors present detailed studies over multiple days to establish assay performance for linearity, accuracy, precision, repeatability, reproducibility, and reusability of the sensor. More extensive validation studies such as this, over a longer period of use, are needed. Progress on these practical issues will be important if affinity biosensors are to achieve their ultimate potential.

In closing, the future looks bright for electrochemical affinity assays/sensors, both academically and commercially. Advances in materials and microfabrication will continue to inspire new approaches for assay and device components. The commercial advantages of the relative simplicity of the actual electrochemical device (no light source or photodetector required and ease of miniaturization) and sensitivity that enables very small samples are strong drivers for commercial products. It should be remembered that 30 years ago, most home testing for blood glucose was done with a spectroscopic-based instrument. This has been displaced by electrochemical sensors

that analyze only a fraction of a drop of blood. Continuous-monitoring implanted devices attached to the skin have recently been implemented, which is a very beneficial advance for the user. Such wearable sensors have exciting potential, and electrochemical affinity sensors are poised to play a major role, as evidenced by the immunosensor for cortisol (153). It took almost 60 years from the seminal paper on the electrochemical glucose biosensor in 1962 (25) by Leland Clark (often called the father of biosensors) and C. Lyons to finally achieve the holy grail of glucose biosensing: continuous personal monitoring of glucose. Electrochemical affinity assays/sensors began in the late 1970s; it will be interesting to see where the field will be in its sixtieth year, about two decades from now.

## DISCLOSURE STATEMENT

P.T.K. is a shareholder of and consultant to BASi Instruments, a manufacturer of electroanalytical instruments, and a founder of Phlebotics, Inc., which is focused on automated blood sampling for point-of-care testing in hospital intensive care units. W.R.H. is a shareholder in BASi Instruments. None of the approaches discussed in this review are used or sold by BASi Instruments. K.R.W. and R.J.W. are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge support from US National Science Foundation grant 1706489 (W.R.H.) and support from the National Institute of General Medical Sciences of the National Institutes of Health under award number R01GM117159 (R.J.W.). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

## LITERATURE CITED

1. Alizadeh N, Salimi A. 2018. Ultrasensitive bioaffinity electrochemical sensors: advances and new perspectives. *Electroanalysis* 30:2803–40
2. Lawal AT. 2018. Progress in utilisation of graphene for electrochemical biosensors. *Biosens. Bioelectron.* 106:149–78
3. Niu XH, Cheng N, Ruan X, Du D, Lin Y. 2020. Nanozyme-based immunosensors and immunoassays: recent developments and future trends. *J. Electrochem. Soc.* 167:037508
4. Sánchez A, Villalonga A, Martínez-García G, Parrado C, Villalonga R. 2019. Dendrimers as soft nanomaterials for electrochemical immunosensors. *Nanomaterials* 9:1745
5. Globe Newswire. 2019. Global immunoassay market is set to reach USD 26.9 billion by 2024, growing at a CAGR of 5.6% during the forecast period: VynZ Research. *Globe Newswire*, July 9. <https://www.globenewswire.com/news-release/2019/07/09/1879881/0/en/Global-Immunoassay-Market-is-Set-to-Reach-USD-26-9-Billion-by-2024-Growing-at-a-CAGR-of-5-6-During-the-Forecast-Period-VynZ-Research.html>
6. Berson SA, Yalow RS. 1959. Quantitative aspects of the reaction between insulin and insulin-binding antibody. *J. Clin. Investig.* 38:1996–2016
7. Zhao H, Boyd LF, Schuck P. 2017. Measuring protein interactions by optical biosensors. *Curr. Protoc. Protein Sci.* 17:20.2.1–22
8. Kirste R, Rohrbaugh N, Bryan I, Bryan Z, Collazo R, Ivanisevic A. 2015. Electronic biosensors based on III-nitride semiconductors. *Annu. Rev. Anal. Chem.* 8:149–69
9. Skládal P. 2016. Piezoelectric biosensors. *TrAC Trends Anal. Chem.* 79:127–33



10. DeMiguel-Ramos M, Diaz-Duran B, Escolano JM, Olivares J, Clement M, et al. 2016. Effects of biologically compatible buffers on the electrical response of gravimetric sensors operating at GHz frequencies. *Sens. Actuators B* 222:688–92
11. Felix FS, Angnes L. 2018. Electrochemical immunosensors—a powerful tool for analytical applications. *Biosens. Bioelectron.* 102:470–78
12. Kokkinos C, Economou A, Prodromidis MI. 2016. Electrochemical immunosensors: critical survey of different architectures and transduction strategies. *TrAC Trends Anal. Chem.* 79:88–105
13. Wen W, Yan X, Zhu C, Du D, Lin Y. 2017. Recent advances in electrochemical immunosensors. *Anal. Chem.* 89:138–56
14. Engvall E, Perlmann P. 1971. Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. *Immunochemistry* 8:871–74
15. Cho IH, Lee J, Kim J, Kang MS, Paik JK, et al. 2018. Current technologies of electrochemical immunosensors: perspective on signal amplification. *Sensors* 18:207
16. Filik H, Avan AA. 2019. Nanostructures for nonlabeled and labeled electrochemical immunosensors: Simultaneous electrochemical detection of cancer markers: a review. *Talanta* 205:120153
17. Zhang Y, Wei Q. 2016. The role of nanomaterials in electroanalytical biosensors: a mini review. *J. Electroanal. Chem.* 781:401–9
18. Breyer B, Radcliff FJ. 1951. Polarographic investigation of the antigen-antibody reaction. *Nature* 167:79
19. Heineman WR, Anderson CW, Halsall HB. 1979. Immunoassay by differential pulse polarography. *Science* 204:865–66
20. Weber SG, Purdy WC. 1979. Homogeneous voltammetric immunoassay: a preliminary study. *Anal. Lett.* 12:1–9
21. Doyle MJ, Halsall HB, Heineman WR. 1982. Heterogeneous immunoassay for serum proteins by differential pulse anodic stripping voltammetry. *Anal. Chem.* 54:2318–22
22. Eggers HM, Halsall HB, Heineman WR. 1982. Enzyme immunoassay with flow-amperometric detection of NADH. *Clin. Chem.* 28:1848–51
23. Heineman WR, Halsall HB. 1985. Strategies for electrochemical immunoassay. *Anal. Chem.* 57:1321A–31A
24. Wehmeyer KR, Doyle MJ, Wright DS, Eggers HM, Halsall HB, Heineman WR. 1983. Liquid-chromatography with electrochemical detection of phenol and NADH for enzyme-immunoassay. *J. Liquid Chromatogr.* 6:2141–56
25. Clark LC Jr., Lyons C. 1962. Electrode systems for continuous monitoring in cardiovascular surgery. *Ann. N. Y. Acad. Sci.* 102:29–45
26. Aizawa M, Morioka A, Matsuoka H, Suzuki S, Nagamura Y, et al. 1976. An enzyme immunosensor for IgG. *J. Solid-Phase Biochem.* 1:319–28
27. Mattiasson B, Nilsson H. 1977. An enzyme immunoelectrode. *FEBS Lett.* 78:251–54
28. Janata J. 1975. Immunoelectrode. *J. Am. Chem. Soc.* 97:2914–16
29. Yamamoto N, Nagasawa Y, Sawai M, Sudo T, Tsubomura H. 1978. Potentiometric investigations of antigen-antibody and enzyme-enzyme inhibitor reactions using chemically modified metal-electrodes. *J. Immunol. Methods* 22:309–17
30. Alexander PW, Rechnitz GA. 1974. Ion-electrode based immunoassay and antibody-antigen precipitin reaction monitoring. *Anal. Chem.* 46:1253–57
31. Ronkainen-Matsuno NJ, Thomas JH, Halsall HB, Heinemann WR. 2002. Electrochemical immunoassay moving into the fast lane. *TrAC Trends Anal. Chem.* 21:213–25
32. Cousino MA, Jarbawi TB, Halsall HB, Heineman WR. 1997. Peer reviewed: pushing down the limits of detection: molecular needles in a haystack. *Anal. Chem.* 69:544A–49A
33. Laocharoensuk R. 2016. Development of electrochemical immunosensors towards point-of-care cancer diagnostics: clinically relevant studies. *Electroanalysis* 28:1716–29
34. Ricci F, Volpe G, Micheli L, Palleschi G. 2007. A review on novel developments and applications of immunosensors in food analysis. *Anal. Chim. Acta* 605:111–29
35. Skládal P. 2019. Advances in electrochemical immunosensors for pathogens. *Curr. Opin. Electrochem.* 14:66–70

36. Cho EJ, Lee JW, Ellington AD. 2009. Applications of aptamers as sensors. *Annu. Rev. Anal. Chem.* 2:241–64
37. Yarman A, Kurbanoglu S, Jetzschmann KJ, Ozkan SA, Wollenberger U, Scheller FW. 2018. Electrochemical MIP-sensors for drugs. *Curr. Med. Chem.* 25:4007–19
38. Kaushik M, Khurana S, Mehra K, Yadav N, Mishra S, Kukreti S. 2018. Emerging trends in advanced nanomaterials based electrochemical genosensors. *Curr. Pharm. Des.* 24:3697–709
39. Akiba U, Anzai JI. 2016. Recent progress in electrochemical biosensors for glycoproteins. *Sensors* 16:2045
40. Xiao T, Huang J, Wang D, Meng T, Yang X. 2020. Au and Au-based nanomaterials: synthesis and recent progress in electrochemical sensor applications. *Talanta* 206:120210
41. Tang J, Tang D. 2015. Non-enzymatic electrochemical immunoassay using noble metal nanoparticles: a review. *Microchim. Acta* 182:2077–89
42. Hou L, Tang Y, Xu M, Gao Z, Tang D. 2014. Tyramine-based enzymatic conjugate repeats for ultrasensitive immunoassay accompanying tyramine signal amplification with enzymatic biocatalytic precipitation. *Anal. Chem.* 86:8352–58
43. Alizadeh N, Salimi A, Hallaj R. 2020. Hemin/G-Quadruplex horseradish peroxidase-mimicking DNzyme: principle and biosensing application. *Adv. Biochem. Eng. Biotechnol.* 170:85–86
44. Bodulev OL, Sakharov IY. 2020. Isothermal nucleic acid amplification techniques and their use in bioanalysis. *Biochemistry* 85:147–66
45. Wan Y, Su Y, Zhu X, Liu G, Fan C. 2013. Development of electrochemical immunosensors towards point of care diagnostics. *Biosens. Bioelectron.* 47:1–11
46. Fernández Abedul MT, González García MB, Costa García A. 2015. Electrochemical immunosensors. In *Agricultural and Food Electroanalysis*, ed. A Escarpa, MC Gonzalez, MA Lopez, pp. 223–93. Chichester, UK: John Wiley & Sons
47. Rama EC, Costa-Garcia A. 2016. Screen-printed electrochemical immunosensors for the detection of cancer and cardiovascular biomarkers. *Electroanalysis* 28:1700–15
48. Niwa O, Xu Y, Halsall HB, Heineman WR. 1993. Small-volume voltammetric detection of 4-aminophenol with interdigitated array electrodes and its application to electrochemical enzyme immunoassay. *Anal. Chem.* 65:1559–63
49. Pei X, Zhang B, Tang J, Liu B, Lai W, Tang D. 2013. Sandwich-type immunosensors and immunoassays exploiting nanostructure labels: a review. *Anal. Chim. Acta* 758:1–18
50. Shrivastava S, Jadon N, Jain R. 2016. Next-generation polymer nanocomposite-based electrochemical sensors and biosensors: a review. *TrAC Trends Anal. Chem.* 82:55–67
51. Fowler JM, Wong DK, Halsall HB, Heineman WR. 2008. Recent developments in electrochemical immunoassays and immunosensors. In *Electrochemical Sensors, Biosensors and Their Biomedical Applications*, ed. X Zhang, HX Ju, J Wang, pp. 115–43. San Diego, CA: Academic
52. Gan SD, Patel KR. 2013. Enzyme immunoassay and enzyme-linked immunosorbent assay. *J. Investig. Dermatol.* 133:e12
53. Amiri M, Bezaatpour A, Jafari H, Boukherroub R, Szunerits S. 2018. Electrochemical methodologies for the detection of pathogens. *ACS Sens.* 3:1069–86
54. Chen C, Luo J, Li C, Ma M, Yu W, et al. 2018. Molecularly imprinted polymer as an antibody substitution in pseudo-immunoassays for chemical contaminants in food and environmental samples. *J. Agric. Food Chem.* 66:2561–71
55. Schoukroun-Barnes LR, Macazo FC, Gutierrez B, Lottermoser J, Liu J, White RJ. 2016. Reagentless, structure-switching, electrochemical aptamer-based sensors. *Annu. Rev. Anal. Chem.* 9:163–81
56. Yu XW, Yang YP, Dikici E, Deo SK, Daunert S. 2017. Beyond antibodies as binding partners: the role of antibody mimetics in bioanalysis. *Annu. Rev. Anal. Chem.* 10:293–320
57. Svalova TS, Malysheva NN, Kozitsina AN. 2017. Structure of the receptor layer in electrochemical immunosensors. Modern trends and prospects of development. *Russ. Chem. Bull. Int. Ed.* 66:1797–811
58. Herne TM, Tarlov MJ. 1997. Characterization of DNA probes immobilized on gold surfaces. *J. Am. Chem. Soc.* 119:8916–20
59. Li H, Dauphin-Ducharme P, Arroyo-Currás N, Tran CH, Vieira PA, et al. 2017. A biomimetic phosphatidylcholine-terminated monolayer greatly improves the in vivo performance of electrochemical aptamer-based sensors. *Angew. Chem. Int. Ed.* 56:7492–95

60. Shaver A, Curtis SD, Arroyo-Currás N. 2020. Alkanethiol monolayer end groups affect the long-term operational stability and signaling of electrochemical, aptamer-based sensors in biological fluids. *ACS Appl. Mater. Interfaces* 12:11214–23
61. Kissinger PT, Heineman WR, eds. 1996. *Laboratory Techniques in Electroanalytical Chemistry*. New York: Marcel Dekker
62. Chang B, Park S. 2010. Electrochemical impedance spectroscopy. *Annu. Rev. Anal. Chem.* 3:207–29
63. Forster RJ, Bertonecello P, Keyes TE. 2009. Electrogenerated chemiluminescence. *Annu. Rev. Anal. Chem.* 2:359–85
64. Santos-Cancel M, Lazenby RA, White RJ. 2018. Rapid two-millisecond interrogation of electrochemical, aptamer-based sensor response using intermittent pulse amperometry. *ACS Sens.* 3:1203–9
65. Bard AJ, Whitesides GM. 1993. *Luminescent metal chelate labels and means for detection*. US Patent 5,238,808A
66. Kuwana T, Strojek. 1967. Optically transparent electrodes. *Am. Chem. Soc. Div. Fuel Chem.* 11:81–84
67. Pan M, Gu Y, Yun Y, Li M, Jin X, Wang S. 2017. Nanomaterials for electrochemical immunosensing. *Sensors* 17:1041
68. Feng T, Wang Y, Qiao X. 2017. Recent advances of carbon nanotubes-based electrochemical immunosensors for the detection of protein cancer biomarkers. *Electroanalysis* 29:662–75
69. Yang H, Xu W, Liang X, Yang Y, Zhou Y. 2020. Carbon nanotubes in electrochemical, colorimetric, and fluorimetric immunosensors and immunoassays: a review. *Microchim. Acta* 187:206
70. Soleymani L, Fang Z, Sargent EH, Kelley SO. 2009. Programming the detection limits of biosensors through controlled nanostructuring. *Nat. Nanotechnol.* 4:844–48
71. Soleymani L, Fang Z, Sun X, Yang H, Taft BJ, et al. 2009. Nanostructuring of patterned microelectrodes to enhance the sensitivity of electrochemical nucleic acids detection. *Angew. Chem. Int. Ed.* 48:8457–60
72. De Luna P, Mahshid SS, Das J, Luan B, Sargent EH, et al. 2017. High-curvature nanostructuring enhances probe display for biomolecular detection. *Nano Lett.* 17:1289–95
73. Huo X, Liu X, Liu J, Sukumaran P, Alwarappan S, Wong DKY. 2016. Strategic applications of nanomaterials as sensing platforms and signal amplification markers at electrochemical immunosensors. *Electroanalysis* 28:1730–49
74. Ren K, Wu J, Zhang Y, Yan F, Ju H. 2014. Proximity hybridization regulated DNA biogate for sensitive electrochemical immunoassay. *Anal. Chem.* 86:7494–99
75. Jain R, Jadon N, Pawaiya A. 2017. Polypyrrole based next generation electrochemical sensors and biosensors: a review. *TrAC Trends Anal. Chem.* 97:363–73
76. Hasanzadeh M, Shadjou N, de la Guardia M. 2018. Nanosized hydrophobic gels: advanced supramolecules for use in electrochemical bio- and immunosensing. *TrAC Trends Anal. Chem.* 102:210–24
77. Liu B, Zhang B, Chen G, Yang H, Tang D. 2014. Proximity ligation assay with three-way junction-induced rolling circle amplification for ultrasensitive electronic monitoring of concanavalin A. *Anal. Chem.* 86:7773–81
78. Zhu Y, Wang H, Wang L, Zhu J, Jiang W. 2016. Cascade signal amplification based on copper nanoparticle-reported rolling circle amplification for ultrasensitive electrochemical detection of the prostate cancer biomarker. *ACS Appl. Mater. Interfaces* 8:2573–81
79. Zhou J, Lai W, Zhuang J, Tang J, Tang D. 2013. Nanogold-functionalized DNzyme concatamers with redox-active intercalators for quadruple signal amplification of electrochemical immunoassay. *ACS Appl. Mater. Interfaces* 5:2773–81
80. Badihi-Mossberg M, Buchner V, Rishpon J. 2007. Electrochemical biosensors for pollutants in the environment. *Electroanalysis* 19–20:2015–28
81. Vogiazzi V, de la Cruz A, Mishra S, Shanov V, Heineman WR, Dionysios DD. 2019. A comprehensive review: development of electrochemical biosensors for detection of cyanotoxins in freshwater. *ACS Sens.* 4:1151–73
82. Bahadır EB, Sezgintürk MK. 2015. Applications of electrochemical immunosensors for early clinical diagnostics. *Talanta* 132:162–74
83. Catanante G, Rhouati A, Hayat A, Marty JL. 2016. An overview of recent electrochemical immunosensing strategies for mycotoxins detection. *Electroanalysis* 28:1750–63

84. Yanez-Sedeno P, Campuzano S, Pingarron JM. 2017. Multiplexed electrochemical immunosensors for clinical biomarkers. *Sensors* 17:965
85. Radecka H, Radecki J. 2015. Label-free electrochemical immunosensors for viruses and antibodies detection—review. *J. Mex. Chem. Soc.* 59:269–75
86. Zhang H, Miller BL. 2019. Immunosensor-based label-free and multiplex detection of influenza viruses: state of the art. *Biosens. Bioelectron.* 141:111476
87. Duffy GF, Moore EJ. 2017. Electrochemical immunosensors for food analysis: a review of recent developments. *Anal. Lett.* 50:1–32
88. Hosu O, Selvolini G, Marrazza G. 2018. Recent advances of immunosensors for detecting food allergens. *Curr. Opin. Electrochem.* 10:149–56
89. Arya SK, Estrela P. 2018. Recent advances in enhancement strategies for electrochemical ELISA-based immunoassays for cancer biomarker detection. *Sensors* 18:2010
90. Filik H, Avan AA. 2020. Electrochemical immunosensors for the detection of cytokine tumor necrosis factor alpha: a review. *Talanta* 211:120758
91. Freitas M, Nouws HPA, Delerue-Matos C. 2018. Electrochemical biosensing in cancer diagnostics and follow-up. *Electroanalysis* 30:1584–603
92. Feng LN, Bian ZP, Peng J, Jiang F, Yang G, et al. 2012. Ultrasensitive multianalyte electrochemical immunoassay based on metal ion functionalized titanium phosphate nanospheres. *Anal. Chem.* 84:7810–15
93. Putnin T, Ngamaroonchote A, Wiriyaun N, Ounnunkad K, Laocharoensuk R. 2019. Dually functional polyethylenimine-coated gold nanoparticles: a versatile material for electrode modification and highly sensitive simultaneous determination of four tumor markers. *Microchim. Acta* 186:305
94. Wang D, Li T, Gan N, Zhang H, Long N, et al. 2015. Electrochemical coding for multiplexed immunoassays of biomarkers based on bio-based polymer-nanotags. *Electrochim. Acta* 163:238–45
95. Wang J. 2012. Electrochemical biosensing based on noble metal nanoparticles. *Microchim. Acta* 177:245–70
96. Chen M, Gan N, Zhang H, Yan Z, Li T, et al. 2016. Electrochemical simultaneous assay of chloramphenicol and PCB72 using magnetic and aptamer-modified quantum dot-encoded dendritic nanotracers for signal amplification. *Microchim. Acta* 183:1099–106
97. Freitas M, Nouws HPA, Keating E, Fernandes VC, Delerue-Matos C. 2020. Immunomagnetic bead-based bioassay for the voltammetric analysis of the breast cancer biomarker HER2-ECD and tumour cells using quantum dots as detection labels. *Microchim. Acta* 187:184
98. Kokkinos C, Angelopoulou M, Economou A, Prodromidis M, Florou A, et al. 2016. Lab-on-a-membrane foldable devices for duplex drop-volume electrochemical biosensing using quantum dot tags. *Anal. Chem.* 88:6897–904
99. Kokkinos C, Economou A. 2017. Emerging trends in biosensing using stripping voltammetric detection of metal-containing nanolabels—a review. *Anal. Chim. Acta* 961:12–32
100. Tang D, Ren J, Lu M. 2017. Multiplexed electrochemical immunoassay for two immunoglobulin proteins based on Cd and Cu nanocrystals. *Analyst* 142:4794–800
101. Zhang Y, Li X, Di YP. 2020. Fast and efficient measurement of clinical and biological samples using immunoassay-based multiplexing systems. *Methods Mol. Biol.* 2102:129–47
102. Fang Y, Huang X, Zeng Q, Wang L. 2015. Metallic nanocrystallites-incorporated ordered mesoporous carbon as labels for a sensitive simultaneous multianalyte electrochemical immunoassay. *Biosens. Bioelectron.* 73:71–78
103. Zhao C, Wu J, Ju H, Yan F. 2014. Multiplexed electrochemical immunoassay using streptavidin/nanogold/carbon nanohorn as a signal tag to induce silver deposition. *Anal. Chim. Acta* 847:37–43
104. Chen X, Ma Z. 2014. Multiplexed electrochemical immunoassay of biomarkers using chitosan nanocomposites. *Biosens. Bioelectron.* 55:343–49
105. Li B, Pu W, Xu H, Ge L, Kwok HF, Hu L. 2019. Magneto-controlled flow-injection device for electrochemical immunoassay of alpha-fetoprotein on magnetic beads using redox-active ferrocene derivative polymer nanospheres. *Analyst* 144:1433–41
106. Shan J, Ma Z. 2016. Simultaneous detection of five biomarkers of lung cancer by electrochemical immunoassay. *Microchim. Acta* 183:2889–97

Downloaded from www.annualreviews.org.

Guest (guest)

IP: 3.147.8.145

On: Sun, 30 Jun 2024 15:26:52

107. Wang J, Munir A, Li Z, Zhou HS. 2010. Aptamer-Au NPs conjugates-accumulated methylene blue for the sensitive electrochemical immunoassay of protein. *Talanta* 81:63–67
108. Zhang B, Liu B, Liao J, Chen G, Tang D. 2013. Novel electrochemical immunoassay for quantitative monitoring of biotoxin using target-responsive cargo release from mesoporous silica nanocontainers. *Anal. Chem.* 85:9245–52
109. Zhao X, Wang J, Chen H, Xu H, Bai L, et al. 2019. A multiple signal amplification based on PEI and rGO nanocomposite for simultaneous multiple electrochemical immunoassay. *Sens. Actuators B* 301:127071
110. Fan C, Plaxco KW, Heeger AJ. 2003. Electrochemical interrogation of conformational changes as a reagentless method for the sequence-specific detection of DNA. *PNAS* 100:9134–37
111. Xiao Y, Lubin AA, Heeger AJ, Plaxco KW. 2005. Label-free electronic detection of thrombin in blood serum by using an aptamer-based sensor. *Angew. Chem. Int. Ed.* 44:5456–59
112. Schoukroun-Barnes LR, Wagan S, White RJ. 2014. Enhancing the analytical performance of electrochemical RNA aptamer-based sensors for sensitive detection of aminoglycoside antibiotics. *Anal. Chem.* 86:1131–37
113. White RJ, Plaxco KW. 2010. Exploiting binding-induced changes in probe flexibility for the optimization of electrochemical biosensors. *Anal. Chem.* 82:73–76
114. Arroyo-Currás Ny, Dauphin-Ducharme P, Ortega G, Ploense KL, Kippin TE, Plaxco KW. 2018. Subsecond-resolved molecular measurements in the living body using chronoamperometrically interrogated aptamer-based sensors. *ACS Sens.* 3:360–66
115. Arya SK, Kongsuphol P, Park MK. 2017. On-chip electrochemical immunoassay platform for specific protein biomarker estimation in undiluted serum using off-surface membrane matrix. *Biosens. Bioelectron.* 91:721–27
116. El-Moghazy AY, Huo J, Amaly N, Vasylieva N, Hammock BD, Sun G. 2020. An innovative nanobody-based electrochemical immunosensor using decorated nylon nanofibers for point-of-care monitoring of human exposure to pyrethroid insecticides. *ACS Appl. Mater. Interfaces* 12:6159–68
117. Eletxigerra U, Martinez-Perdiguerro J, Merino S. 2015. Disposable microfluidic immuno-biochip for rapid electrochemical detection of tumor necrosis factor alpha biomarker. *Sens. Actuators B* 221:1406–11
118. Jović M, Zhu Y, Lesch A, Bondarenko A, Cortés-Salazar F, et al. 2017. Inkjet-printed microtiter plates for portable electrochemical immunoassays. *J. Electroanal. Chem.* 786:69–76
119. Montiel VRV, Campuzano S, Torrente-Rodríguez RM, Reviejo AJ, Pingarrón JM. 2016. Electrochemical magnetic beads-based immunosensing platform for the determination of  $\alpha$ -lactalbumin in milk. *Food Chem.* 213:595–601
120. Nam EJ, Kim EJ, Wark AW, Rho S, Kim H, Lee HJ. 2012. Highly sensitive electrochemical detection of proteins using aptamer-coated gold nanoparticles and surface enzyme reactions. *Analyst* 137:2011–16
121. Ocana C, Hayat A, Mishra R, Vasilescu A, del Valle M, Marty JL. 2015. A novel electrochemical aptamer-antibody sandwich assay for lysozyme detection. *Analyst* 140:4148–53
122. Rackus DG, Dryden MDM, Lamanna J, Zaragoza A, Lam B, et al. 2015. A digital microfluidic device with integrated nanostructured microelectrodes for electrochemical immunoassays. *Lab Chip* 15:3776–84
123. Xu T, Zhang H, Li X, Xie Z, Li X. 2015. Enzyme-triggered tyramine-enzyme repeats on prussian blue-gold hybrid nanostructures for highly sensitive electrochemical immunoassay of tissue polypeptide antigen. *Biosens. Bioelectron.* 73:167–73
124. Feng J, Li Y, Li M, Li F, Han J, et al. 2017. A novel sandwich-type electrochemical immunosensor for PSA detection based on PtCu bimetallic hybrid (2D/2D) rGO/g-C<sub>3</sub>N<sub>4</sub>. *Biosens. Bioelectron.* 91:441–48
125. Chang BY, Park SM. 2010. Electrochemical impedance spectroscopy. *Annu. Rev. Anal. Chem.* 3:207–29
126. Guo X, Kulkarni A, Doepke A, Halsall HB, Iyer S, Heineman WR. 2012. Carbohydrate-based label-free detection of *Escherichia coli* ORN 178 using electrochemical impedance spectroscopy. *Anal. Chem.* 84:241–46
127. Khetani S, Kollath V, Kundra V, Nguyen M, Debert C, et al. 2018. Polyethylenimine modified graphene-oxide electrochemical immunosensor for the detection of glial fibrillary acidic protein in central nervous system injury. *ACS Sens.* 3:844–51
128. Ramanathan M, Patil M, Epur R, Yun Y, Shanov V, et al. 2016. Gold-coated carbon nanotube electrode arrays: Immunosensors for impedimetric detection of bone biomarkers. *Biosens. Bioelectron.* 77:580–88

129. Shamsipur M, Farzin L, Tabrizi MA, Molaabasi F. 2015. Highly sensitive label free electrochemical detection of VEGF165 tumor marker based on “signal off” and “signal on” strategies using an anti-VEGF165 aptamer immobilized BSA-gold nanoclusters/ionic liquid/glassy carbon electrode. *Biosens. Bioelectron.* 74:369–75
130. Blonder R, Levi S, Tao G, Ben-Dov I, Willner I. 1997. Development of amperometric and microgravimetric immunosensors and reversible immunosensors using antigen and photoisomerizable antigen monolayer electrodes. *J. Am. Chem. Soc.* 119:10467–78
131. Ramsey JM, Jacobson SC, Knapp MR. 1995. Microfabricated chemical measurement systems. *Nat. Med.* 1:1093–95
132. Bange A, Halsall HB, Heineman WR. 2005. Microfluidic immunosensor systems. *Biosens. Bioelectron.* 20:2488–503
133. Choi J-W, Vijayawardhana CA, Okulan N, Oh KW, Han A, et al. 2000. Development and characterization of a generic microfluidic subsystem toward portable biochemical detection. In *Micro Total Analysis Systems*, ed. A van den Berg, W Olthuis, P Bergveld, pp. 327–30. Dordrecht, Neth.: Springer
134. Choi J-W, Oh KW, Thomas JH, Heineman WR, Halsall HB, et al. 2002. An integrated microfluidic biochemical detection system for protein analysis with magnetic bead-based sampling capabilities. *Lab Chip* 2:27–30
135. Wang J, Ibanez A, Chatrathi MP, Escarpa A. 2001. Electrochemical enzyme immunoassays on microchip platforms. *Anal. Chem.* 73:5323–27
136. Heineman WR, Halsall HB, Seliskar CJ, Aguilar ZP, Farrell S, et al. 2007. *Detection of Bioterrorism Agents in Water Supplies*. Denver, CO: AWWA Res. Found., Am. Water Works Assoc.
137. Tang J, Tang D, Niessner R, Chen G, Knopp D. 2011. Magneto-controlled graphene immunosensing platform for simultaneous multiplexed electrochemical immunoassay using distinguishable signal tags. *Anal. Chem.* 83:5407–14
138. Bai H, Bu S, Wang C, Ma C, Li Z, et al. 2020. Sandwich immunoassay based on antimicrobial peptide-mediated nanocomposite pair for determination of *Escherichia coli* O157:H7 using personal glucose meter as readout. *Microchim. Acta* 187:220
139. Boonkaew S, Chaiyo S, Jampasa S, Rengpipat S, Siangproh W, Chailapakul O. 2019. An origami paper-based electrochemical immunoassay for the C-reactive protein using a screen-printed carbon electrode modified with graphene and gold nanoparticles. *Microchim. Acta* 186:153
140. Hasanzadeh M, Shadjou N. 2016. Electrochemical and photoelectrochemical nano-immunesensing using origami paper based method. *Mater. Sci. Eng. C* 61:979–1001
141. Kwon D, Joo J, Lee S, Jeon S. 2013. Facile and sensitive method for detecting cardiac markers using ubiquitous pH meters. *Anal. Chem.* 85:12134–37
142. Ma C, Liu H, Zhang L, Li L, Yan M, et al. 2017. Microfluidic paper-based analytical device for sensitive detection of peptides based on specific recognition of aptamer and amplification strategy of hybridization chain reaction. *Chem. ElectroChem.* 4:1744–49
143. Martinez AW, Phillips ST, Whitesides GM. 2010. Diagnostics for the developing world: microfluidic paper-based analytical devices. *Anal. Chem.* 82:3–10
144. Teengam P, Siangproh W, Tontisirin S, Jiraseree-amornkun A, Chuaypen N, et al. 2021. NFC-enabling smartphone-based portable amperometric immunosensor for hepatitis B virus detection. *Sens. Actuators B* 326:128825
145. Dungchai W, Chailapakul O, Henry CS. 2009. Electrochemical detection for paper-based microfluidics. *Anal. Chem.* 81:5821–26
146. Ozer T, McMahon C, Henry CS. 2020. Advances in paper-based analytical devices. *Annu. Rev. Anal. Chem.* 13:85–109
147. Ye R, Zhu C, Song Y, Lu Q, Ge X, et al. 2016. Bioinspired synthesis of all-in-one organic-inorganic hybrid nanoflowers combined with a handheld pH meter for on-site detection of food pathogen. *Small* 12:3094–100
148. Tang D, Zhang B, Liu B, Chen G, Lu M. 2014. Digital multimeter-based immunosensing strategy for sensitive monitoring of biomarker by coupling an external capacitor with an enzymatic catalysis. *Biosens. Bioelectron.* 55:255–58

Downloaded from www.annualreviews.org.

Guest (guest)

IP: 3.147.8.145

On: Sun, 30 Jun 2024 15:26:52

149. Teymourian H, Parrilla M, Sempionatto JR, Montiel NF, Barfidokht, et al. 2020. Wearable electrochemical sensors for the monitoring and screening of drugs. *ACS Sens.* 5:2679–700
150. Bandodkar AJ, Jeang WJ, Ghaffari R, Rogers JA. 2019. Wearable sensors for biochemical sweat analysis. *Annu. Rev. Anal. Chem.* 12:1–22
151. Yu Y, Nyein HYY, Gao W, Javey A. 2019. Flexible electrochemical bioelectronics: the rise of in situ bioanalysis. *Adv. Mater* 32:e1902083
152. Tu J, Torrente-Rodriguez RM, Wang M, Gao W. 2020. The era of digital health: a review of portable and wearable affinity biosensors. *Adv. Funct. Mater.* 30:1906713
153. Parlak O, Keene ST, Marais A, Curto VF, Salleo A. 2018. Molecularly selective nanoporous membrane-based wearable organic electrochemical device for noninvasive cortisol sensing. *Sci. Adv.* 4:eaar2904
154. Heyrovsky J. 1922. Elektrolýsa se rtuťovou kapkovou katódou. *Chem. Listy* 16:256–64
155. Medetalibeyoglu H, Beytur M, Akyidirim O, Atar N, Yola ML. 2020. Validated electrochemical immunosensor for ultra-sensitive prolactin detection: carbon electrode modified with gold nanoparticles functionalized sulfur doped MXene as sensor platform and carboxylated graphitic carbon nitride as signal amplification. *Sens. Actuators B* 319:128195
156. Lin P-H, Li B-R. 2020. Antifouling strategies in advanced electrochemical sensors and biosensors. *Analyst* 145:1110–20
157. Russo MJ, Han M, Desroches PE, Manasa CS, Dennaoui J, et al. 2021. Antifouling strategies for electrochemical biosensing: mechanisms and performance toward point of care based diagnostic applications. *ACS Sens.* 6:1482–507